

**IL-17RA signaling in oral epithelium is necessary and sufficient for protection against oropharyngeal candidiasis**

Conti *et al.*

**SUPPLEMENTAL INFORMATION**

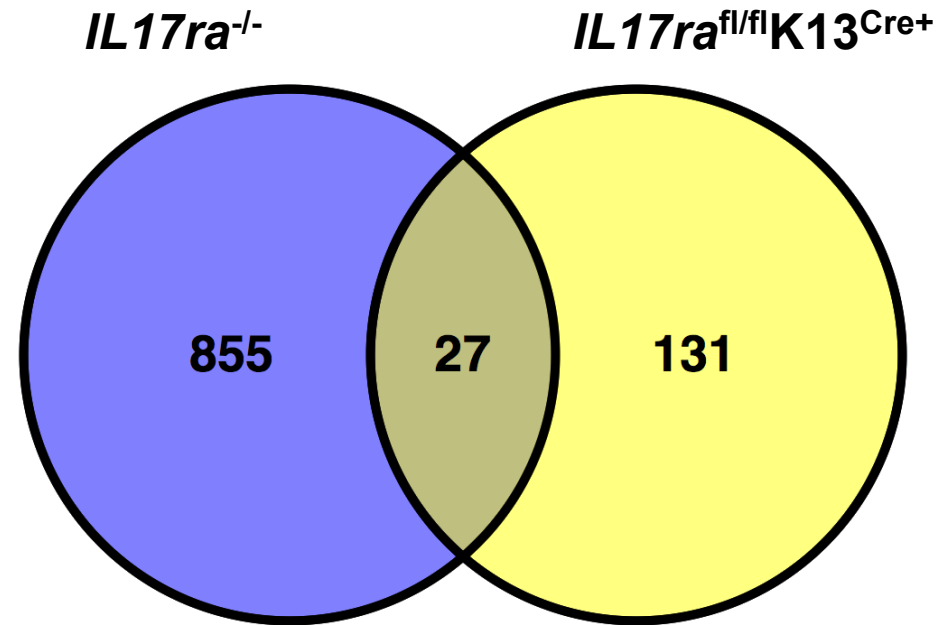
**Figure S1, related to Figure 6A.** Common and unique transcriptional responses of each infected mutant, *Il17ra*<sup>-/-</sup> or *Il17ra*<sup>fl/fl</sup> K13<sup>Cre+</sup> relative to response of infected *Il17ra*<sup>fl/fl</sup> K13<sup>Cre-</sup> (WT) mice. A differentially expressed gene is defined as having an absolute fold change of 2 or greater, P<0.05. Refer to Table S2 for gene names and expression values.

**Table S1, related to Figure 6A.** Values represent the log (base 2) fold change of (pre-treated with IL-17 +TNF and infected)/(no pre-treatment and infected). “Inf” indicates that no reads were detected in the naïve samples but p-value criteria were met.

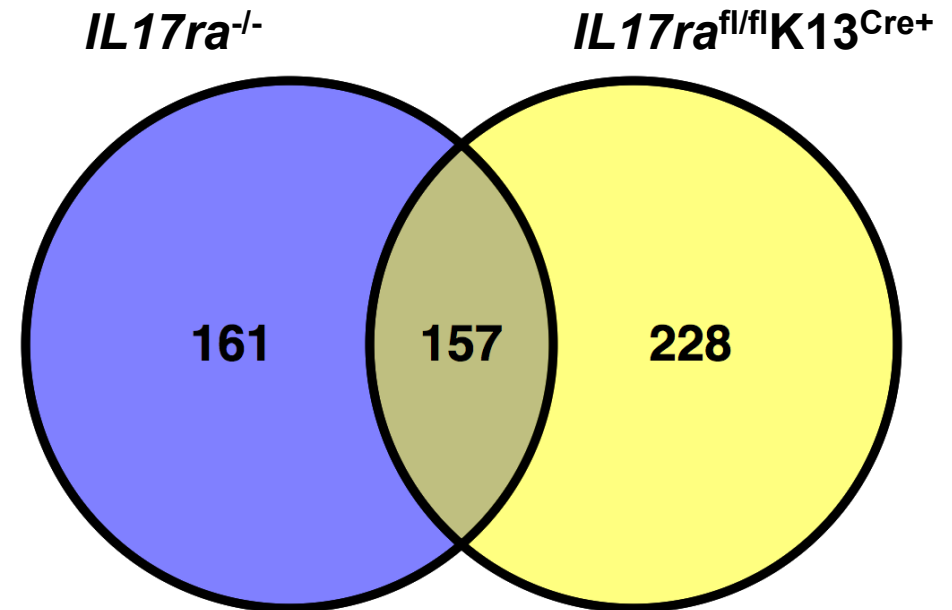
**Table S2, related to Figure 6A.** Values represent the log (base 2) fold change of (1st group/2nd group). “Inf” indicates that no reads were detected in the 2nd group but the p-value criteria were met. “-Inf” indicates that no reads were detected in the 1st group but the p-value criteria were met. “0” indicates that no statistically significant difference in gene expression was observed. All comparisons include data from 3 mice per group. This table contains all of the genes that were differentially expressed in at least 1 of the 3 comparisons.

Fig S1 (Conti et al.)

Genes with **increased** expression in mutant relative to wild-type (infected mutant/ infected Wild-type)



Genes with **decreased** expression in mutant relative to wild-type (infected mutant/ infected Wild-type)



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### **Detailed Experimental Procedures**

#### ***Cell culture***

OKF6/TERT2 cells (Dickson et al., 2000), provided by J. Rheinwald (Brigham & Women's Hospital, Boston MA), were cultured in Serum-Free Fibroblast media, 25 µg/ml bovine pituitary extract and 2 ug/ml EGF (Life Technologies, Grand Island NY). Cells were treated with TNFα (0.5 ng/mL) and IL-17A (50-200 ng/mL) (R&D Systems) for 24 h and then infected with  $1 \times 10^7$  *C. albicans* yeast for 5 h. TR146 cells were obtained from ECACC (Salisbury, United Kingdom) and cultured in DMEM-F12/15% FBS.

#### ***K13 promoter***

The murine *Krt13* promoter (nucleotides -3090 to +40) was obtained by PCR from the BAC clone RP23-10A2. Primers used were: 5'-GCTTAGTGGGTAGCAAGCTTG-3'; 5'-GCAGACAGGAGCTGAACTTGAC-3'. This fragment was subcloned into the pGL3-basic vector in the NarI and SbfI restriction sites (Promega). Activity was assessed in IMOK cells transfected with FuGENE 6 and normalized to a pCMV-LacZ control vector (Parikh et al., 2008). Luc activity was assessed using the Luciferase Assay system (Promega), and β-galactosidase with the Galacton Plus kit (Life Technologies).

## ***Mice***

*Il17ra*<sup>-/-</sup> mice and anti-IL-17RA Abs were a gift from Amgen (Seattle WA). *Il17ra*<sup>fl/fl</sup> mice were made as described (Kumar et al., 2016). *Defb3*<sup>-/-</sup> mice were from the MMMRC (UC Davis, CA). To create K13<sup>LacZ</sup> mice, the 3.1 kb *Krt13* promoter was subcloned into the pNASSβ expression vector (Promega). The K13-LacZ cassette was isolated and microinjected into pronuclei of F2 (B10xC3H) fertilized oocytes at the Roswell Park Cancer Institute (RPCI) Transgenic Core Facility. Xgal staining of oral tissue sections to detect β-galactosidase activity was performed on 2 independent lines of K13<sup>LacZ</sup> transgenic mice, with similar results (Romano et al., 2010). K13<sup>CRE</sup> transgenic mice were created with the *Krt13*-NLS-Cre cassette microinjected into fertilized C57BL/6 oocytes by the Pittsburgh Transgenic and Gene Targeting Core Facility, and founders identified by PCR (5'-TCAAGTTCAGCTCCTGTCTG-3'; 5'-TCGCGAACATCTTCAGGTTC-3'). Two (of 4) founders showed expression profiles similar to the K13<sup>LacZ</sup> mice, and subsequent experiments used line #139. C57BL/6 and *Gt(Rosa)26<sup>tm1Sor</sup>* mice were from The Jackson Laboratory (Bar Harbor ME). Experiments were performed in accordance with IACUC protocols approved by the University of Pittsburgh, the State University of New York at Buffalo or RPCI.

## ***Oral Candidiasis***

OPC was performed by sublingual inoculation with a 0.0025 mg cotton ball saturated in *C. albicans* (CAF2-1) for 75 min as described (Solis and Filler, 2012). Tongues were homogenized in PBS for fungal load enumeration or frozen in liquid nitrogen for mRNA analysis. Tongue homogenates were dissociated on a GentleMACS (Miltenyi Biotec, Cambridge MA) and serial dilutions plated on YPD+Amp agar. All efforts were made to minimize suffering,

in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH.

### ***Salivary assays***

Saliva (100  $\mu$ l) was collected by pipet following i.p. carbachol injection (10  $\mu$ g/ml) and used immediately in candidacidal assays (Conti et al., 2009). 90  $\mu$ l of saliva was incubated with  $10^4$  cells of *C. albicans* in 10  $\mu$ l PBS for 1 h at 37°C, plated in triplicate and assayed after 2 d for CFU enumeration on YPD-Amp. Data were normalized to PBS alone controls.

### ***Immunohistochemistry, ELISA, Lactate dehydrogenase cytotoxicity assays***

Cryosections were stained with  $\alpha$ -IL-17RA mAbs (Amgen),  $\alpha$ -MPO mAbs (R&D Systems) or anti-rat BD3 Abs (Santa Cruz Biotechnology) using the BioLegend IHC Protocol for Frozen Tissue or Immunocruz LSAB Staining System. Briefly, slides were fixed in acetone and blocked for 1 h in 10% rat or goat serum. Slides were incubated with 1° Abs in 0.5% BSA/PBS for 18 h at 4°C followed by biotinylated 2° Ab. SA-HRP conjugates were used followed by DAB substrate (BD Biosciences). Sections were counter-stained with hematoxylin, acid washed ( $\alpha$ -MPO and  $\alpha$ -BD3) and cleared with xylene. Images were captured with an Evos FL Auto microscope (Life Technologies). Image analysis was performed by 2 independent assessors in a blinded fashion. BD2 ELISAs were performed with Abs from Peprotech. LDH assays were performed with a CytoTox 96 Assay System in triplicate (Promega).

### ***RNA-Seq and qPCR***

RNA-Seq libraries were prepared with total RNA using a Qubit 2.0 fluorometer (Thermo Fisher)

and Agilent Bioanalyzer TapeStation 2200 (Agilent Technologies). Strand specificity was achieved using dUTP in the Second Strand Marking Mix, followed by cDNA synthesis with DNA Polymerase I and RNase H. Products were enriched by PCR, and the resulting cDNA library was validated using KAPA Biosystems primer premix kit (KAPA Biosystems) with Illumina-compatible DNA primers and Qubit 2.0 fluorometer. Quality was verified by Agilent TapeStation. Cluster generation and 75 bp single read single-indexed sequencing was performed on Illumina NextSeq 500. For OKF6-TERT2 *in vitro* infections, RNA-seq libraries (non-strand specific, paired-end) were prepared with the TruSeq RNA kit (Illumina). RNA was subjected to poly(A) enrichment by the TruSeq protocol. 100 nucleotides were determined from each end of cDNA fragments using the HiSeq platform.

Raw sequencing reads (75bp) were checked for sequencing issues and contaminants with FastQC (Babraham Bioinformatics). Adapter sequences, primers, Ns, and reads with quality score <28 were trimmed using fastq-mcf of ea-utils and PRINSEQ. Single reads were aligned to the UCSC mouse or human reference genomes (mm10, GRCm38.75; Ensembl GRCh38) using TopHat2. Differential gene expression was assessed using DESeq (Bioconductor). Read coverage was computed using SAMtools, BEDtools, and UCSC Genome Browser. Pairwise differential expression was quantified with Cuffdiff. Cufflinks was used to determine FPKM levels for each gene from the STAR alignment and was used as input for Cuffdiff. Read counts were then normalized across all samples and differentially expressed genes were determined by adjusted *P*-value with a threshold of 0.05.

For real-time PCR, RNA was isolated from tongue with RNeasy Mini Kits (QIAGEN), and cDNA generated with Superscript III First Strand Kits (Invitrogen). Real-time qPCR using SYBR Green FastMix ROX (Quanta Biosciences) was performed using a 7300 Real time

instrument (Applied Biosystems) and expression normalized to GAPDH. Primers were from Superarray Biosciences or QuantiTect Primer Assays (QIAGEN).

### ***Data Access***

Upon acceptance, raw sequencing reads will be submitted to the NCBI Sequence Read Archive (SRA; <http://www.ncbi.nlm.nih.gov/sra>) and processed gene expression data will submitted to the NCBI Gene Expression Omnibus (GEO). The RNA-seq data for the in vitro infections has been deposited under accession code SRP077728.

### **References**

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